THE DEVELOPMENT OF THE NUCLEI OF THE SPINNING-GLAND CELLS OF PLATYPHYLAX DESIGNATUS WALKER (TRICHOPTERON).

C. T. VORHIES.

The large and complexly branched nuclei of the spinning-gland cells of trichoptera (and lepidoptera) contain two kinds of stainable material. That one kind of this material is chromatin there is no doubt; that the other is nucleolar material perhaps the majority of cytologists now believe, but there have been some differences of opinion concerning this point. These materials in the glands of trichoptera — at least in *P. designatus* and some other species — consist of larger, irregularly shaped masses, and small, more evenly sized granules, which have a roughened appearance as if made up of smaller particles. In the lepidopteron, *Isia isabella*, the two materials are scarcely distinguishable by the size of particles, but only by their staining reactions. The smaller granules stain blue in the triple stain of Flemming, while the larger take the red stain characteristic of nucleolar material. These larger particles will be hereinafter referred to as nucleoles or nucleolar material.

A brief review of the literature of this subject seems desirable.

Helm (1876), though he indicates the ontogenetic changes of form of the nuclei, does not distinguish the contents of the same.

Carnoy (1884), in a rather diagrammatic drawing (Fig. 54) of a spinning-gland cell from the larva of a trichopter, shows filaments of "nucléine" which undoubtedly represent the nucleolar material. He does not distinguish the chromatin in this drawing. In Figs. 78 and 79 he shows a portion of a branched nucleus from the gland of a moth larva, and a cell from the gland of the larva of a microlepidopter. In both of these, the nucleolar material and chromatin are indicated, but the chromatin is not mentioned and the nucleolar material is designated as the "boyau nucléinien." This skein of nuclein he believes to be

made up in its turn as in a nucleus — a quite incorrect view, of course, as we know it. In his Fig. 132, *A* and *B*, he indicates the difference in form of the nuclei from young and old larvæ of a microlepidopter.

Gilson (1890) figures sections of spinning-gland nuclei of lepidoptera, but does not distinguish the character of the nuclear contents, nor does he discuss the nature of the material. The same author (1894) distinguishes the two materials in glands of trichoptera. He mentions the parts as follows: "Disons seulement qu'il existe au sein de l'amas de tronçons nucléiniens, des corps arrondis, chromatique, des nucléoles particuliers qui souvent laissent voir dans leurs interieure des cordons nucléiniens, semblables à ceux qui constituent la grande masse du contenu nucléaire."

Korschelt (1896), working on various species of lepidoptera, used a modification of the Ehrlich-Biondi stain. He found the larger particles (macrosomes) to stain green, the smaller ones (microsomes) to stain red, and concluded that the macrosomes must be regarded as chromatin, the microsomes as nucleoli.

Meves (1897), employing various methods of staining, shows that the microsomes of Korschelt are chromatin granules, and that the macrosomes of that author must be regarded as nucleoli. He employed Heidenhain's formula of the Ehrlich-Biondi stain and got the opposite result to that of Korschelt, who used a formula with the methyl green much stronger.

Korschelt (1897) upholds the view previously expressed by him as to the nature of the macrosomes and microsomes.

Flemming (1897) agrees with Meves.

Henneguy (1904), p. 463, refers to the conclusions of Korschelt and Meves and records his researches as agreeing with the latter.

Marshall and Vorhies (1906) conclude as the result of the use of various stains, that the larger bodies are nucleoles, the smaller granules chromatin.

It is evident from the above review that all of our evidence upon the nature of the stainable materials is that derived from the staining reactions. While with our present knowledge of such reactions the proof thus offered is very good, yet the presence of such large amounts of nucleolar material as are found in the

spinning-gland nuclei and the fact, as shown by Korschelt's work, that changes in the formulæ of certain stains may give conflicting results, it seems very desirable that evidence of a somewhat different kind be obtained, if possible. It was with the object of ontogenetic evidence in view that the present work was begun.

It may be remarked at the outset that it was not anticipated that the task would prove quite so easy of accomplishment as it has, since it was supposed that the later embryonic stages would require investigation. This proved not to be the case, however, the earliest stages necessary to solve the problem being the young larvæ as they emerged from the egg.

Methods. — It was found that the spinning-glands must be dissected or teased out before fixation to secure the best results, since even the youngest larvæ are not penetrated readily by the fluids. Decapitation gives quite good results, as some parts of the glands will then usually protrude from the cut end of the body. The most satisfactory method found for the smallest larvæ was to plunge them alive into the fixative, then turning the ventral side up, and holding the posterior end down with a needle, the head can be caught with the point of another needle and pulled off. The glands, not being fastened posteriorly, will in most cases draw cleanly out of the body. With larger larvæ this is not easy of accomplishment, and decapitation is the better method. With the largest larvæ, dissection from the dorsal side while immersed in the fixative is best. Flemming's strong formula, Tower's solution and 95 per cent. alcohol were most used, as fixing agents. As described in previous papers, Vorhies (1905), Marshall and Vorhies (1906), Delafield's hæmatoxylin was most satisfactory for whole mounts of the glands. The method of splitting the glands previously described cannot, of course, be used with the glands from larvæ only 1.5 mm. to 2 mm. in length, but the difficulties to be overcome by splitting in the larger glands are in these not so great, therefore it does not matter. For sections, Flemming's triple stain, and iron hæmatoxylin were used almost exclusively. Both sections and whole mounts were made of the various stages, the whole mounts serving as a useful check on the sections, and to show the changes in form of the nuclei.

At the time of hatching the larvæ of *P. designatus* are about

1.5 mm. in length. Glands from such larvæ show very clearly the two rows of cells forming the wall of the gland, and the addition of a little dilute methyl green to a drop of normal salt solution containing them shows at once that the nuclei are of a simple, unbranched type: typically the nuclei are round at this stage, though a few are slightly elongated. It may be noted here that from the time of breaking out of the egg membrane to the time of emergence from the mass of jelly containing the eggs may be some hours for an individual, and 24 hours barely suffices for a brood to get clear of the jelly after the first ones are seen moving within the mass. To distinguish carefully in every case between those larvæ fresh from the egg membrane and those just out of the jelly would be rather more laborious than results would warrant, so in speaking of larvæ just hatched I mean larvæ in the jelly or out of it only a few hours.

In each of the round or slightly elongated nuclei of this first period there is, almost without exception, a single nucleole present (Figs. 1 and 2). This nucleole is large, round or elongated, smooth in outline, lies near the center of the nucleus, takes the stains characteristic of the nucleoles of ordinary cells, and, in short, is undoubtedly a true nucleolus. A study of fresh glands of larvæ just out of the egg or even yet within it, shows that the elongated (rarely divided) nucleoles belong generally to the larvæ which have been for some time out of the egg.

Larvæ which have been out of the jelly for twenty-four hours, and which have been supplied with sand, possess a well constructed case. Glands from such larvæ contain a larger proportion of elongated nuclei, the elongation being transverse to the long axis of the gland. In whole preparations there may be found single round or elongated nucleoles, and, in many instances, two or three nucleoles in one nucleus (Figs. 3, 5, 6). Sections at this stage show that the two nucleoles in a nucleus arise *by division of the original one*, since various stages of elongation and constriction, giving more or less dumb-bell-shaped figures may be found. By division here is not meant bipartition necessarily, but fragmentation, since evidences of the latter process are readily observable (Fig. 4). From this time on the nucleoles are more ragged and irregular in appearance, but this may

be due to the metabolic activity of the gland (Marshall and Vorhies, 1906).

On account of the difficulty of keeping the larvæ in the laboratory the exact ages of the larvæ with reference to the following events have not been obtained, but that is scarcely necessary for the purpose of this work. With glands from larger and larger larvæ, more and more of the nuclei are found to contain two nucleoles, occasionally three or four are present (Figs. 7, 8), and in larvæ about one week old, and 2 mm. or more in length (which may have molted) as many as 4–7 nucleoles are typically present in each nucleus (Figs. 8, 9). The nucleoles now increase in number continuously as the nuclei increase in size (Figs. 10–14), and, except that they become somewhat more uneven in size and more irregular in shape, there is little to be noted. A more detailed account with drawings showing their characteristics in various large nuclei is contained in the 1906 paper already referred to.

The changes in form of the nuclei, already briefly mentioned, consist first in a lengthening in the direction of the circumference of the gland (Figs. 2, 4–7); in a larva one week old, the nuclei are two or three times as long as they are wide (Figs. 7–9). It will be noted that there is a marked increase in number of nucleoles before there is any great change in shape of the nuclei (Figs. 9, 10). Swellings next appear, which elongate and develop into branches (Fig. 11). Since there are no distinct centers of branching (Vorhies, 1905), as figured by Henneguy (1904), there is no regular order of development to be traced. At first, it appears that a majority of the elongated nuclei have two branches at one end forming a T-figure, or two at each end, forming a kind of modified H-figure (Fig. 12), but if there really is any such tendency the increasing complexity of the branch system soon obscures it. The complexity simply increases with the increase in size of the cell (Figs. 13, 14). There does not, however, appear to be a high degree of correlation between the size of the cell and the space occupied within it by the nucleus (Marshall and Vorhies, 1906, Figs. 1–6).

The condition of the chromatin remains the same, so far as its staining reaction and appearance are concerned, throughout the

nuclear history as outlined. The granules in the large nuclei are of about the same actual size, with the same inter-granular spaces, as in the young rounded nuclei. No evidence was noted in the staining reaction which might lead to the conclusion that those of the younger nuclei were merely more dense. Indeed, the difference in size of the nuclei is so great that it is almost impossible to conceive of the chromatin of one of the large nuclei being compressed or condensed to the relatively few granules found in a small one. The chromatin must therefore increase in amount. In all stages a linin reticulum is easily distinguishable with the higher magnifications, particularly in sections.

Conclusions.

1. The larger particles of stainable material in the spinning-gland nuclei of *P. designatus* are derived by division and growth from an original nucleole of normal type and hence may be regarded as true nucleoles.

2. The red-staining granules in the nuclei of similar glands of lepidoptera, whether larger than the chromatin granules or not, are probably of similar origin and character.

3. This nucleolar material increases in amount with the growth of the nucleus.

4. The latter conclusion coupled with one in a former paper (Marshall and Vorhies, 1906, p. 417), that the nucleoles become irregular as a result of glandular activity, leads to the further conclusion that the nucleolar material bears a direct relation to such activity: whether as a waste product or as a material functional in secretion I make no assertion.[1]

5. The chromatin increases in amount with the growth of the

[1] In connection with conclusion 4, attention may be called to the following from Montgomery (1899), p. 537. "The hypothesis might be suggested that though the nucleolus probably consists of substances which stand in some relation to the nutritive processes of the nucleus, and so at the time of its formation may be a functionless, inert mass of substance, yet it may at later periods in the history of the resting nucleus acquire some active function and thus gradually come to acquire the value of a nuclear organ; this hypothesis is put forward merely as a tentative one. According to this view the nucleolus might be considered as an organ which serves to accumulate in itself the waste products of the nucleus, thus serving as a reservoir for such substances; or it might be considered as an organ of excretion, to discharge waste products out of the nucleus; in either case the nucleolus would seem to stand in direct connection with the nutritive substances and forces of the nucleus."

nucleus, and therefore probably has a functional part in the process of secretion. The interesting question now arises as to whether the material which thus increases for this purpose is identical with the material which we believe to be the bearer of the hereditary qualities, or whether it is of a different nature functionally, but with the same staining reaction. This must remain an open question. There seems to the writer no reason why the same material cannot determine the direction of development of the cell (*i. e.*, carry hereditary qualities) and also determine the functional activity of the cell after its differentiation.

In connection with the last two conclusions it may be well to note that it has been repeatedly stated that in the process of yolk formation in eggs, cytoplasm (yolk) is formed from substance which is given off from the nucleus as buds (Blochmann, Scharff, Balbiani), as extrusions of parts of the chromatin (Fol, Blochmann, Van Bambeke, Erlanger, Mertens, Calkins), or as nucleolar substance (Leydig, Balbiani, Will, Henneguy): also that Miss Huie (1897) working on gland cells of *Drosera*, and Mathews (1899) working on pancreas cells, got analogous results with those secretory cells.

UNIVERSITY OF WISCONSIN,
MADISON, WIS.

LITERATURE.

Carnoy, J. B.
'84 La biologie cellulaire. Etude comparee de la cellule dans les deux regnes. Paris.

Flemming, W.
'97 Zelle. Merkel und Bonnet's Ergebn. d. Anat. u. Entw., Bd. 5.

Gilson, G.
'90 Recherches sur les cellules secretantes. I. Lepidopteres. La Cellule, T. VI.
'96 Recherches sur les cellules secretantes. II. Trichopteres. La Cellule, T. X.

Henneguy, L.
'04 Les Insectes. P. 463. Paris.

Huie, L.
'97 Changes in the Cell-organs of *Drosera* Produced by Feeding with Egg-albumen. Quart. Jour. Micr. Sci., Vol. XXXIX.

Korschelt, E.
'96 Ueber die Struktur der Kerne in den Spinndrüsen der Raupen. Archiv f. mikr. Anat., Bd. XLVII.
'97 Ueber den bau der Kerne in den Spinndrüsen der Raupen. Archiv f. mikr. Anat., Bd. XLIX.

Marshall, W. S., and Vorhies, C. T.

'06 Cytological Studies on the Spinning-glands of *Platyphylax designatus* Walker (Phryganid). Internat. Monatsschrift f. Anat. u. Physiol., Bd. XXIII.

Mathews, A. P.

'99 The Metabolism of the Pancreas Cell. Jour. Morph., XV., Suppl.

Meves, F.

'97 Zur Struktur der Kerne in den Spinndrüsen der Raupen. Archiv f. mikr. Anat., Bd. XLVIII.

Montgomery, T. H., Jr.

'99 Comparative Cytological Studies, with Especial Regard to the Morphology of the Nucleolus. Jour. Morph., XV.

Vorhies, C. T.

'05 Habits and Anatomy of the Larva of the Caddis-fly, *Platyphylax designatus* Walker. Trans. Wis. Acad. Sci. Arts and Letters, Vol. XV., Part I.

Explanation of Plate I.

All nuclei figured which are longer in one dimension than in the other lie with the long axis transverse to the long axis of the gland.

Fig. 1. Nucleus of a spinning-gland cell from a larva just hatched; the single nucleolus is typical in size, shape and position. × 1,300.

Fig. 2. A nucleus from the same gland as that in Fig. 1. Nucleolus elongated. × 1,300.

Fig. 3. Nucleus from a larva "just hatched" but probably a little older than the one from which Figs. 1 and 2 are taken. × 1,300.

Fig. 4. Nucleus from the same larva as Fig. 3. Nucleole fragmenting. × 1,300.

Fig. 5. Slightly elongated nucleus from same larva as Figs. 3 and 4. Two nucleoles. × 1,300.

Fig. 6. Slightly elongated nucleus from the same larva as Figs. 3, 4 and 5. Two irregular nucleoles. × 1,300.

Fig. 7. Elongated nucleus from a larva twenty-four hours old. Nucleoles irregular, three in number. × 1,300.

Figs. 8 and 9. Larger nuclei from a larva about one week old, showing further fragmentation of the nucleolus. × 1,300.

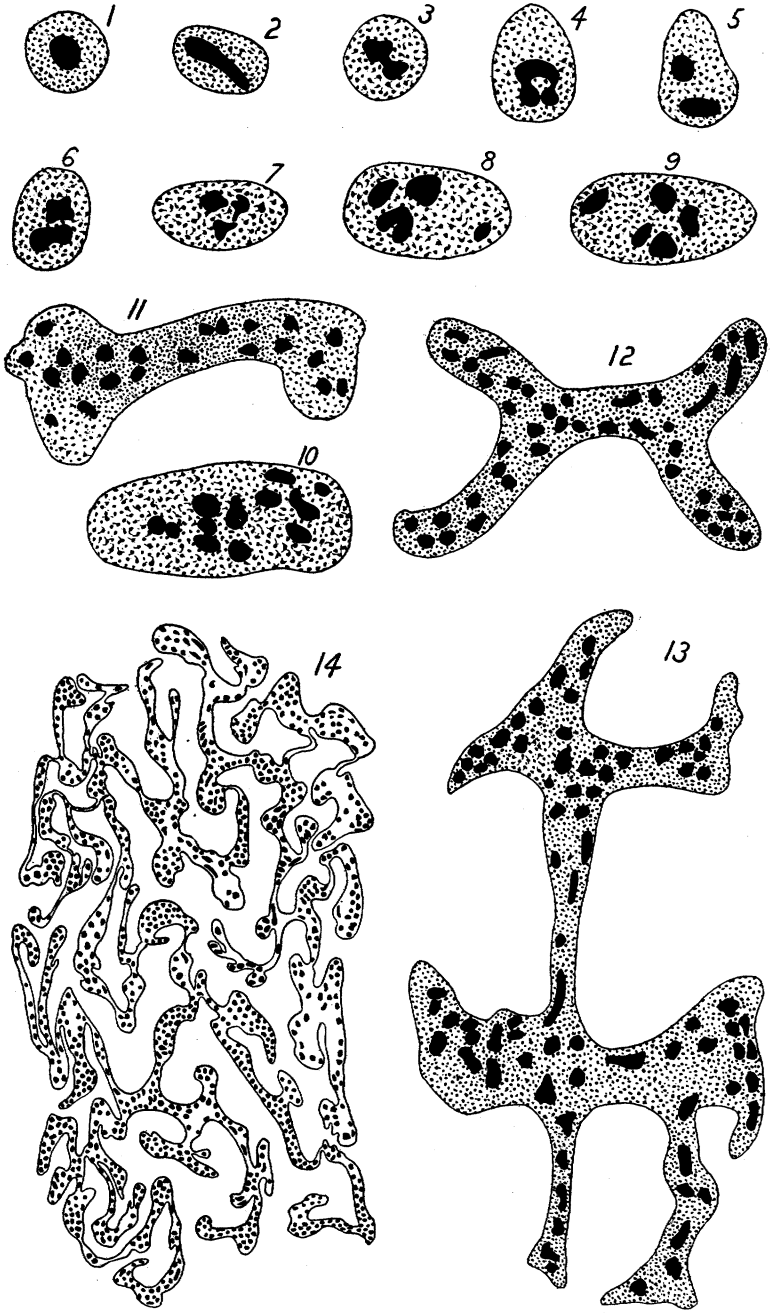
Fig. 10. Nucleus from a larva about one week old, which has probably molted. Twelve nucleoles present. × 1,300.

Fig. 11. Nucleus from a larva about 3 mm. in length. It is probably about two weeks old and has certainly molted. Twenty-two nucleoles. × 800.

Fig. 12. Nucleus from another larva 3 mm. in length. Many nucleoles. Ends of the nucleus branching. × 800.

Fig. 13. Nucleus from a larva 5 mm. in length. Branching more complex, and nucleoles more numerous. × 800.

Fig. 14. Nucleus from an adult larva, about 16 mm. in length, containing a very large number of nucleoles. No attempt has been made to represent the chromatin in this figure, as the magnification is not great enough. × 175.


1
2
3
4
5
6
7
8
9
11
12
10
14
13